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On the origin of spontaneous activity in the bladder

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Author contributions.

NK performed the research; CHF designed the research study; NK and CHF analysed the data; NK and CHF wrote the paper

ABSTRACT

Objectives. To characterise separately the pharmacological profiles of spontaneous contractions from the mucosa and detrusor layers of the bladder wall and to describe the relationship in mucosa between ATP release and spontaneous contractions.

Materials and Methods. Spontaneous contractions were measured (36°C) from isolated mucosa or detrusor preparations, and intact (mucosa+detrusor) preparations from guinea-pig bladders. Potential modulators were added to the superfusate. Percentage smooth muscle was measured in haematoxylin and eosin stained sections. ATP release was measured in superfusate samples from a fixed point above the preparation using a luciferin-luciferase assay.

Results. The magnitude of spontaneous contractions was in the order intact>mucosa>detrusor. Percentage smooth muscle was least in mucosa and greatest in detrusor preparations. The pharmacological profiles of spontaneous contractions were different in mucosa and detrusor in response to P2X or P2Y receptor agonists, adenosine and capsaicin. Intact preparations showed responses intermediate to those from mucosa and detrusor preparations. Low extracellular pH generated large changes in detrusor, but not mucosa preparations. Mucosa preparations released ATP in a cyclical manner, followed by variations in spontaneous contractions. ATP release was greater in mucosa compared to detrusor, augmented by carbachol and reversed by the M2-selective antagonist methoctramine.

Conclusions. The different pharmacological profiles of bladder mucosa and detrusor implies different pathways for contractile activation. Intermediate responses from intact preparations also implies functional interaction. The temporal relationship between cyclical variation of ATP release and amplitude of spontaneous contractions is consistent with ATP release controlling spontaneous activity. Carbachol-mediated ATP release was independent of active contractile force.

Abbreviations.

ABMA, α,β -methylene-adenosine triphosphate; ACh, acetylcholine; ADP, adenosine diphosphate; ANOVA; analysis of variance; ATP, adenosine triphosphate; AUC; area under the curve; CCh, carbachol; MES, 2-(*n*-morpholino)ethanesulfonic acid; SC, spontaneous contraction; UDP, uridine diphosphate; UTP, uridine triphosphate

INTRODUCTION

The bladder generates spontaneous contractions, as measured in vivo, in isolated whole organs, or in isolated preparations. Moreover, these contractions are greater with bladders that have outflow tract obstruction or neuropathy from spinal cord damage (1-3). It may be proposed that these phenomena underlie the spontaneous contractions and sensations of urgency associated with the very prevalent condition of overactive bladder syndrome and thus it would be of value to understand their (patho)physiology. Several theories have been proposed for normal and abnormal spontaneous contractions (4) including: continuous transmitter release from motor nerves; intrinsic myogenic activity of detrusor smooth muscle; or an interaction between detrusor smooth muscle and a mucosa, composed of urothelium and sub-urothelium. The latter, urotheliogenic hypothesis (5) asserts that the mucosa, by release of diffusible agents or by intercellular communication via interstitial cells, generates spontaneous contractions. It is given credibility by the fact that when stretched the mucosa releases agents such as ATP and acetylcholine (ACh) (6,7) and spontaneous contractions cease when it is removed from the underlying detrusor (3). In addition some reports show that isolated mucosa preparations also develop spontaneous contractions (8). This raises the possibility that the mucosa itself directly contributes to overall bladder contractions and is especially pertinent as the mucosa thickens in conditions associated with bladder overactivity (9).

However, the contractile characteristics of the mucosa and how it influences detrusor function remain to be characterised fully. This study aimed to describe more fully mucosa contractile activity, how it modifies detrusor function and what may be the role of ATP released from mucosa.

METHODS

Preparations and solutions. Dunkin-Hartley guinea-pigs (350-450 g) were euthanized by procedures approved by the UK Home Office (Animals (Scientific Procedures) Act 1986; Amendment Regulations SI 2012/3039) procedures. The bladder was immediately removed and placed in Ca-free Tyrode's solution and cut open from the base to dome. The bladder sheet was pinned out in a dissection dish filled with Tyrode's solution, mucosal face uppermost and the mucosa itself removed from one half by blunt dissection. The mucosa itself was placed in a separate dissection dish. Strips (< 1mm diameter, 5 mm length) of mucosa and bladder wall (with or without attached mucosa) were dissected with sharp scissors and mounted in a horizontal superfusion trough. They were tied at one end to a fixed hook and at the other to an isometric force transducer mounted on a micromanipulator to adjust resting length. Rapid stretch or relaxation by 0.5 mm was achieved via the micromanipulator. Preparation length, l , and weight, w , were recorded and tension, T , expressed per unit cross-sectional area – radius, a , was calculated from; $a = \sqrt{W/(\rho\pi l)}$, where ρ is muscle density (1.05 g.ml⁻¹).

Preparations were superfused with Tyrode's (37°C) at 3-4 ml.min⁻¹. Tyrode's contained (mM): NaCl, 118; KCl, 4.0; NaHCO₃, 24; NaH₂PO₄, 0.4; MgCl₂, 1.0; CaCl₂, 1.8; glucose, 6.1, Na pyruvate, 5.0; pH 7.4 with 95%O₂/5%CO₂. All chemicals for interventions were stored as stock solutions at least 100-times the final concentration by dilution in Tyrode's. Final concentrations were: ABMA, 1 µM; ADP, UTP and UDP all 30 µM; adenosine, 1 mM; capsaicin, 30 µM - values were determined from previous experiments to exert maximum effects on detrusor and interstitial cells (3,10-13). Low pH solution (Tyrode's-MES) contained 10 mM MES buffered to pH 5.5 with HCl. All chemicals were from Sigma, UK.

Tension recording and analysis. Outputs from the force transducer, via a bridge amplifier, were digitally stored for analysis. Two components of tension output were analysed: i) the magnitude of

tonic tension or relaxation during an intervention, the maximum value was recorded; ii) the integral of spontaneous activity superimposed on baseline or tonic contraction/relaxation tension (area under the curve, AUC) Outputs were analysed for 10-minute periods immediately prior to an intervention, at the beginning or end of an intervention, and immediately or 30-minutes after return to control.

Histology. Sample preparations were placed in 4% paraformaldehyde and stored at 4°C, then dehydrated with increasing ethanol solutions, placed in xylene, and paraffin mounted. Sections (7 µm) were stained with haematoxylin and eosin solution. Digital images (x20) were analysed by ImageJ to quantify the proportional areas of muscle and interstitial space; areas with no staining were not analysed.

ATP measurement. ATP released from the preparation was measured in a 20 µl sample aspirated from a fixed point 100 µm above the centre of the preparation surface. A luciferin-luciferase assay (FL-AAM, Sigma, GloMax 20/20; Promega) was used at either a two- or ten-fold dilution. Ten readings were taken for each sample and the average used. Luminescence was calibrated against [ATP] between 0.1 nM and 1 µM and was linear on a log-log plot, with Tyrode's or Tyrode's-MES used as a blank solution where appropriate. Changes of [ATP] in superfusate samples are expressed as $\Delta p\text{ATP}$ ($\Delta(-\log[\text{ATP}])$) rather than absolute amounts normalised to preparation weight. This is because the ATP quantities in different superfusate samples will reflect proportional changes to [ATP], but not total ATP release, as long as the sampling site remained fixed.

Data analysis and statistics. Data are shown as median values [25, 75% interquartiles]. Differences between data sets were tested by ANOVA, with non-parametric paired or unpaired *post hoc* tests. The association between two variables was tested by calculation of Spearman's rank coefficients, r , and the significance obtained by calculation of a t value by: $t = r\sqrt{(n-2)/(1-r^2)}$, followed by calculation of p -values; n = number of preparations. The null hypothesis was rejected at $p < 0.05$.

Curve-fits (KaleidaGraph, Synergy Inc) were achieved by an iterative least-squares fit program and periodic data were analysed by generating a best-fit Fourier series of multiple sine functions.

RESULTS

Spontaneous contractions. Figure 1A shows sections of the intact bladder wall, detrusor-only and mucosa-only preparations. The intact wall sample shows a urothelium and suburothelium overlaying the deeper detrusor layer. The detrusor preparation consists predominantly of smooth muscle, whereas the mucosa preparation has only diffuse and separated muscle bundles. Examples of spontaneous contractions from intact, and isolated detrusor or mucosa preparations are shown in Figure 1B. Intact preparations developed the largest contraction integral (area under the curve, AUC) per unit cross-section area and detrusor preparations the smallest, with mucosa intermediate (Figure 1C, note the contractile ordinates in this and part E are on a log-axis due to the large differences of values in the three preparations). The average frequencies of contractions in mucosa and intact preparations were 2.3 ± 0.5 vs 2.1 ± 0.8 .min⁻¹, $n=19,15$ respectively, $p>0.05$). Percentage smooth muscle was greatest in detrusor preparations (73.4 [90.1, 72.0]%), smallest in mucosa preparations (4.5 [6.6, 1.9]%) and intermediate in intact preparations (62.8 [73.6, 54.4]%, all $n=10$, Figure 1C). Normalisation of integral contractile activity to muscle cross-section area showed it was about 100-times in mucosa compared to that in detrusor (1.8 [0.46, 5.5] vs 0.024 [0.013, 0.16] mN.min.mm⁻²: $p<0.001$): the value for intact preparations was intermediate (0.21 [0.053, 0.28] mN.min.mm⁻²: $p<0.01$ vs mucosa and detrusor, Figure 1D). In three experiments placing mucosa originally dissected from an intact preparation restored spontaneous activity. This implies that there is a diffusible factor between mucosa and detrusor affecting spontaneous activity and dissection of mucosa from detrusor did not damage either layer when used for subsequent measurement of contractile function. It may be concluded that: i) detrusor alone has little intrinsic ability to generate spontaneous activity; ii) interaction between mucosa and detrusor generates enhanced spontaneous activity; iii) with mucosa preparations either the small muscle component has a greater intrinsic contractility than detrusor, or non-muscle tissue contributes to spontaneous activity.

The hypothesis that spontaneous mucosa contractions had a non-detrusor origin was tested by comparing responses from mucosa and detrusor preparations to different contractile agonists. The same agonists were also tested on intact preparations to measure any interaction between the two layers. Several classes of agents were tested: a P2X-receptor agonist ($\alpha\beta$ -methylene ATP – ABMA); P2Y-receptor agonists (ADP, UTP, UDP); a P1-receptor agonist (adenosine); and capsaicin. The more complex effects of low extracellular pH superfusate are considered separately below.

Comparison of mucosa and detrusor preparations – spontaneous contractions. Figure 2 shows the effect of the above agents on isolated mucosa and detrusor preparations: part A illustrates ABMA or capsaicin. ABMA generated a transient tonic contraction in both preparations, but diminished spontaneous activity (AUC) in mucosa, whilst increasing the small activity in detrusor; portions of the spontaneous activity trace in the detrusor preparation before and during ABMA superfusion have been expanded for clarity. Capsaicin diminished both tonic and spontaneous activity in mucosa, whilst increasing them in detrusor.

Figures 2B and C summarise the effect of these agents, as well as P2Y and P1 agonists, on mucosa and detrusor spontaneous contractions (AUC), respectively. The profile of responses was different in the two preparations. With mucosa ABMA and capsaicin decreased but UDP increased responses, ADP, UTP and adenosine had no significant effects. With detrusor ABMA, UTP and capsaicin all increased responses, whilst it was reduced by adenosine; ADP and UDP had no significant effects.

Comparison of mucosa and detrusor preparations – tonic contractions. P2X and P2Y agonists evoked transient contractures in both mucosa and detrusor preparations (Figure 3A,B). With ABMA the effect was significantly greater in detrusor preparations; with P2Y agonists contractures were not significantly different from mucosa or detrusor. However, adenosine and capsaicin had opposite

effects: adenosine contracted mucosa but relaxed detrusor; capsaicin abolished resting tone in mucosa, even below the base-line, whilst increasing it in detrusor.

Overall, spontaneous contractions and contractures from mucosa and detrusor preparations had different response profiles to a range of contractile agonists suggesting that the contractile activation process was also dissimilar in these two tissues.

Responses of intact tissues – spontaneous and tonic responses. Intact tissue responded to the above agents with a somewhat different profile from mucosa and detrusor alone. Figure 4A illustrates the effects of ADP, UDP and capsaicin. Figure 4B shows that only ABMA and UDP increased spontaneous activity, as with their actions on detrusor and mucosa, respectively. There was no effect of capsaicin, in contrast to the large and opposite effects on mucosa and detrusor alone. Tonic activity had a profile similar to mucosa and detrusor alone for ABMA and P2Y agonists, i.e. an increase (Figure 4C). There was no effect of adenosine and capsaicin, again in contrast to their opposing actions on mucosa and detrusor.

The effect of reduced extracellular pH. Reduction of extracellular pH had complex effects on spontaneous and tonic activity (Fig 5A). The initial effect on spontaneous activity was to reduce mucosa, but increase detrusor activity, and depress tonic activity in both (Fig 5B,C). At longer times detrusor spontaneous and tonic activity increased above the control response, but mucosa activity returned only to control. Immediately on return to normal extracellular pH detrusor preparations generated even greater increases of spontaneous and tonic activity before return to control; mucosa preparations remaining unaffected. Changes to intact preparations largely mirrored those of detrusor alone, except that with spontaneous activity the initial augmentation was absent and subsequent changes were smaller.

Spontaneous contractions and ATP release. Superfusate [ATP] was measured every two minutes and levels were cyclic, a sample experiment is shown in figure 6A. Data were analysed by Fourier transformations to find the frequency and amplitude of the changes. In eight experiments the ATP variations had a frequency of 0.049 [0.048, 0.051] min⁻¹, and amplitude (Δp ATP) 0.30 [0.22,0.40].

Superfusate [ATP], as measured every two minutes, was correlated to the integral of spontaneous contraction activity (AUC) over the preceding two minutes, but the correlation was not significant ($r=0.033$ [-0.035, 0.213], $n=8$ preparations). However, if the time-frame between [ATP] and AUC was varied the correlation improved as AUC increasingly lagged [ATP] values. For a second sample experiment (figure 6B) there was no significant correlation if [ATP] was related to the previous 2-minute AUC – termed lag0 ($r=0.253$; $p=0.344$; top left). If the [ATP] was correlated to 1-3 minutes prior AUC (termed lag-1, top right) the correlation remained non-significant ($r=0.349$; $p=0.128$; top right). However, as the AUC period increasingly lagged behind the [ATP] measurements correlation improved, with lag1 and lag2 correlations being significant ($r=0.672$; $p=0.0045$ and $r=0.817$; $p=0.0002$; bottom left and right, respectively). An interpretation is that changes to AUC result from changes of [ATP]. Figure 6C shows the variation of r -value with time lag for the experiment in figure 6B and fitted by a non-specific third order polynomial: the maximum r -value was 0.846 at a time lag of 2.1 min. From eight experiments the median lag was 2.0 min [1.9, 2.2].

AUC was the most useful index of contractile function; neither frequency nor amplitude alone were consistent as variables between preparations. Moreover, contractions were often fused with others to make estimation of either frequency or amplitude very difficult. However, for illustrative purposes Figure 6D shows an example of spontaneous activity from a mucosa preparation where frequency was fairly constant. The inset shows a plot of the average amplitude of pairs of contractions as a function of time, with a superimposed sinusoid to show variation of amplitude. Such pairing of traces was the most useful method to show temporal fluctuation of amplitude when evident.

Sources of ATP. The preceding data are consistent with the hypothesis that spontaneous ATP release from mucosa influences the magnitude of spontaneous contractions. Artificial stretch of mucosa preparations generated ATP release and was significantly greater than from detrusor preparations (figure 7A). Carbachol (0.1 μ M) also increased ATP release from intact preparations regardless of whether they were stretched or relaxed (figure 7B). Carbachol also induced active contractions that may have themselves contributed to ATP release, however raised KCl (80 mM) which induced equivalent increases of contraction generated only a small ATP release. Pre-treatment with the M₂ receptor-selective antagonist, methoctramine (1 μ M) blocked carbachol-induced ATP release; furthermore, methoctramine itself induced a significant fall of ATP release from resting preparations (figure 7C). Finally, extracellular acidosis to 5.5 had no effect on ATP release during induction of acidosis and on return to normal pH despite large contractile changes (figure 5): Δ pATP values early and late in acidosis were 0.023 [-0.050, 0.127] and 0.006 [-0.002, 0.063]; on early return to control Δ pATP values were 0.039 [-0.043, 0.048] (all, $p > 0.05$ vs control, $n=6$).

DISCUSSION AND CONCLUSIONS

The experiments show that the mucosa is independently capable of generating spontaneous contractions, greater in magnitude than from isolated detrusor preparations. However, when the two bladder wall layers are in contact spontaneous activity is greater than the sum of individual activities, suggesting interaction between the two layers. The study had two major objectives: to determine if the contractile characteristics of mucosa and detrusor were different; and secondly to identify the cause of spontaneous activity in the mucosa. It is concluded that control of spontaneous activity by exogenous agents is different in the two layers and spontaneous mucosa contractions may be driven by variable release of ATP, itself under the control of muscarinic receptor activation.

Mucosa and detrusor contractile characteristics. The pharmacological profiles of mucosa and detrusor spontaneous activity were different: the P2X agonist ABMA and capsaicin had opposite effects, whereas for P2Y-receptor agonists, UDP increased mucosal activity, but in detrusor UTP was effective. With respect to base-line (contracture) tension, adenosine and capsaicin had opposite effects on the two preparations. The differential effects of ADP, UTP and UDP on spontaneous activity provide preliminary insight regarding different functional P2Y receptor subtypes in mucosa and detrusor, although it was not a primary objective here to characterise functional P2Y receptor subtypes in the two layers. However, for P2Y₆ receptors UDP is the most selective agonist (14) and thus they may play a more significant role in mucosa, whereas for P2Y₄ receptors UTP is selective over ADP. UTP is also a natural ligand for P2Y₂, but not for P2Y₁ receptors (14). Thus, detrusor spontaneous activity may be regulated more by P2Y_{2/4} receptors and P2Y₆ receptors have a greater role in mucosa. These data should be followed by using more selective P2Y-receptor subtype agonists and antagonists.

The differential pharmacology of mucosa and detrusor may provide clues to the cells that regulate and contribute to spontaneous activity, particularly in the mucosa. The muscularis mucosa could

contribute to spontaneous contractions (15) and it made up about 8% of the total tissue mass (Figure 1D). Muscularis mucosa contractions are dependent on Ca^{2+} influx pathways as well as Ca^{2+} release from intracellular stores. Furthermore, it has been suggested that because muscularis mucosa may be affected by contractile agonists released from the urothelium, a large proportion of such cells may be recruited, but a smaller proportion of detrusor cells generate force (15). Another source of contractile function could originate from pericytes surrounding the large number of suburothelial venules. These cells develop spontaneous intracellular Ca^{2+} transients dependent on release from intracellular Ca-stores filled by Ca^{2+} influx through store-operated Ca^{2+} channels and are associated with contractions of the venules (16). Finally, in mucosa, interstitial cells are a dominant cell population that are electrically active (17), ADP, UDP and UTP all generate large inward currents and a rise of intracellular $[\text{Ca}^{2+}]$, and P2Y_6 , P2Y_2 and P2Y_4 antibodies label epitopes, most strongly for P2Y_6 (18), consistent with interstitial cells contributing to spontaneous contractions. Interstitial cells can contain contractile proteins with a different form of myosin from smooth muscle, often called non-muscle myosin (19).

Spontaneous contractions from pig and guinea-pig bladder mucosa may rely on local acetylcholine release acting on M3 receptors, are suppressed by nitric oxide and modulated by adrenoreceptor activation (8,18,20). Electrical field stimulation also generates tetrodotoxin-sensitive contractions of isolated mucosa, consistent with a functional control of this contractile activity by motor nerves (21,22). The functional transmitters remain to be identified and the particular cells generating contractions remain to be identified but there is substantial evidence that the mucosa contributes to the contractile activity of the bladder wall.

ATP release and spontaneous contractions. Previous studies with isolated mucosa preparations have associated ATP release to mechanical or agonist-induced increase of tension; however, the causality of the two variables was not defined (23). This study has shown that ATP release precedes

spontaneous contractions, as shown by surges of ATP release occurring before increased spontaneous activity. In most of the preparations ATP release, and thus spontaneous activity, was cyclical with an interval of about 20 minutes and an amplitude of about 30% of the baseline value and this cyclical activity had correlates in spontaneous contractions. The mechanism for cyclical ATP release is not known but the long interval implies that there is a considerable amount of ATP released. Experiments with isolated mucosa strips or urothelial cells show that after maximum ATP release through mechanical stress a period of about 60 minutes is required before further maximal release can be evoked (23,24). This phenomenon, if manifest in an unco-ordinated manner over the entire mucosa of the bladder wall, would generate a basal mechanical tone.

ATP release from mucosa was regulated by muscarinic receptor agonists but was independent of whether the tissue had a resting tension or was flaccid (25). Furthermore, ATP release was not due to tissue contraction, as a high-KCl solution, to generate a comparable contraction to carbachol, was without effect. The lack of effect of high-KCl solution also implies that depolarisation of component cells was not responsible for ATP release with carbachol. This is consistent with abolition of carbachol-mediated ATP release by the M2-selective agent methoctramine as M2 receptor activation mediates cellular effects independent of membrane potential changes. The crucial role of muscarinic receptor agonists to modulate ATP release suggests acetylcholine has a regulatory role under basal conditions, as methoctramine reduced ATP release in the absence of added carbachol.

Conclusions. The detrusor and mucosa layers of the bladder wall are both capable of independent spontaneous contractile activity, but the two layers when in contact enhance overall activity. The contractile characteristics are however different as judged by their responses to a spectrum of purinergic receptor agonists, capsaicin and extracellular pH changes to suggest that contractile activation is different in the two layers. The bladder wall demonstrated cyclical ATP release, primarily from the mucosa. In turn ATP release was controlled by M2 receptor activation,

independent of active or passive tension in the preparation. Moreover, ATP release preceded spontaneous contractile activity, the pathway relating these two phenomena remains to be elucidated.

Limitations. Experiments used *in vitro* preparations and whether these conclusions extrapolate to *ex vivo* or *in vivo* conditions remains to be tested. The functional purinergic receptor subtypes responsible for spontaneous contractile activity were not an objective of this study and remain to be characterised. ATP released from the mucosa will be broken down by endogenous extracellular ATPases, the extent to which this occurs in the *in vitro* preparations used here, as compared to more intact preparations also requires evaluation. Finally, the restoration of spontaneous activity by placing a dissected mucosa strip placed on a detrusor preparation in turn attached to the force transducer implies a role for a diffusible agent from mucosa to detrusor. This study did not determine the causative agent, which will be the subject of the next study, but did demonstrate that the mucosa retains the ability to influence underlying detrusor contraction even after physical connection to the smooth muscle layer has been removed.

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FIGURE LEGENDS

Figure 1. Spontaneous contractile activity from intact bladder wall, detrusor and mucosa isolated preparations. A: Haematoxylin and eosin sections of (left to right): intact strip, isolated detrusor and isolated mucosa preparations. B: experimental traces from the three preparations; note the different calibration bar for the intact preparations. C: Integrated spontaneous activity (area under the curve, AUC) normalised to preparation cross-section area. D: smooth muscle as a percentage of total cross-section area for the three preparations. E: AUC normalised to muscle cross-section area. Note the logarithmic ordinates used for B and D. Data are medians [25%, 75% interquartiles], *n* values in text.

Figure 2. Effect of P2X, P2Y, P1 receptor agonists and capsaicin on spontaneous activity of mucosa and detrusor. A: experimental traces showing effect of ABMA (left) or capsaicin (right) on isolated mucosa (upper traces) and detrusor (lower traces) preparations. Sections of the detrusor ABMA trace have been enlarged five-fold before and after addition of the agent to highlight the increase of spontaneous activity in amplitude in the presence of ABMA. B: effects of ABMA (*n*=6), P2Y agonists (ADP, UTP, UDP; *n*=6,6,9), adenosine (aden, *n*=6) and capsaicin (capsc, *n*=8) on AUC of mucosa spontaneous contractions with respect to control (=100%, dotted line). C: effect of ABMA (*n*=7), P2Y agonists (ADP, UTP, UDP; *n*=7,6,6), adenosine (*n*=6) and capsaicin (*n*=7) on AUC of detrusor spontaneous contractions with respect to control (=100%). Median [25%, 75% interquartiles]; **p* <0.05 vs control.

Figure 3. Effect of P2X, P2Y, P1 receptor agonists and capsaicin on contractures from mucosa and detrusor. A: effects of ABMA ($n=6$), P2Y agonists (ADP, UTP, UDP; $n=6,6,9$), adenosine (aden, $n=6$) and capsaicin (capsc, $n=8$) on mucosa preparations with respect to control (=100%, dotted line). B: effect of ABMA ($n=7$), P2Y agonists (ADP, UTP, UDP; $n=7,6,6$), adenosine ($n=6$) and capsaicin ($n=7$) on detrusor preparations with respect to control (=100%). Median [25%, 75% interquartiles]; $*p < 0.05$ vs control.

Figure 4. Effect of P2X, P2Y, P1 receptor agonists and capsaicin on spontaneous activity and contractures of intact preparations. A: experimental traces showing effect of ADP, UDP and capsaicin. B: effect of ABMA ($n=5$), P2Y agonists (ADP, UTP, UDP; $n=7$), adenosine (aden, $n=8$) and capsaicin (capsc, $n=8$) on AUC of spontaneous contractions with respect to control (=100%, dotted line). C: effect of ABMA, P2Y agonists, adenosine and capsaicin on contracture (baseline) tension respect to control (=100%, n values as in part B). Median [25%, 75% interquartiles]; $*p < 0.05$ vs control.

Figure 5. Effect of low pH on contractile activity of mucosa, detrusor and intact preparations. A: experimental traces showing effect of low pH solution on the three preparations. B: AUC of mucosa (M) and detrusor (D) spontaneous contractions for 0-10 minutes (early) and 20-30 (late) minutes of exposure to low pH solution, and 0-10 minutes on return to control (early washout). C: contracture of mucosa (M) and detrusor (D) preparations for early and late periods of exposure to low pH solution, and at early washout. Median [25%, 75% interquartiles]; $n=6$; $*p < 0.05$ vs control.

Figure 6. The relationship between released ATP and AUC of spontaneous contractions from mucosa preparations. A: Variation of ATP levels as a function of time. The line is a best-fit polynomial fit to the data but has no special significance. B: The temporal relationship between AUC and ATP release. Top left, ATP associated to two previous minutes of AUC (lag 0); top right, ATP associated to AUC in 1-3 minutes prior to ATP sampling (lag -1); bottom left, ATP associated to AUC in two minutes spanning ATP sampling time (lag 1)' bottom right, ATP is associated to AUC two minutes after ATP sampling time (lag 2). C: The relationship between lag-time and the correlation coefficient (*r*-value) for the ATP-AUC relationship. The line is a three-powered polynomial to the data for estimation of the lag period at which the *r*-value is maximised. D: A recording of spontaneous activity from a mucosa preparation. The inset shows variation of contraction amplitude (successive pairs of contractions are averaged). The sine wave is a best-fit with equation: $Tension = (0.05 * \sin(0.31x)) + 0.24$, where *x* is the first contraction number of successive pairs (see text for details).

Figure 7. The effect of physical and chemical interventions on ATP release from isolated mucosa. A: ATP release ($\Delta pATP$) from detrusor (*n*=4) and mucosa (*n*=10) preparations, **p*<0.05 mucosa vs detrusor. B: ATP release induced by carbachol (CCh) from relaxed (*n*=8) or stretched (*n*=8) intact preparations, stretched preparations in the presence of carbachol (*n*=4) and high-KCl solution and high-KCl solution alone (*n*=4). Median [25%, 75% interquartiles]; * *p* <0.05 vs KCl vs CCh stretched. C: ATP release from relaxed preparations by carbachol (CCh) in the absence or presence of methoctramine (*n*=4), and in the presence of methoctramine alone (*n*=4). Median [25%, 75% interquartiles]; #*p*<0.05 CCh vs methoctramine+CCh, **p*<0.05 methoctramine vs control.

Figure 1

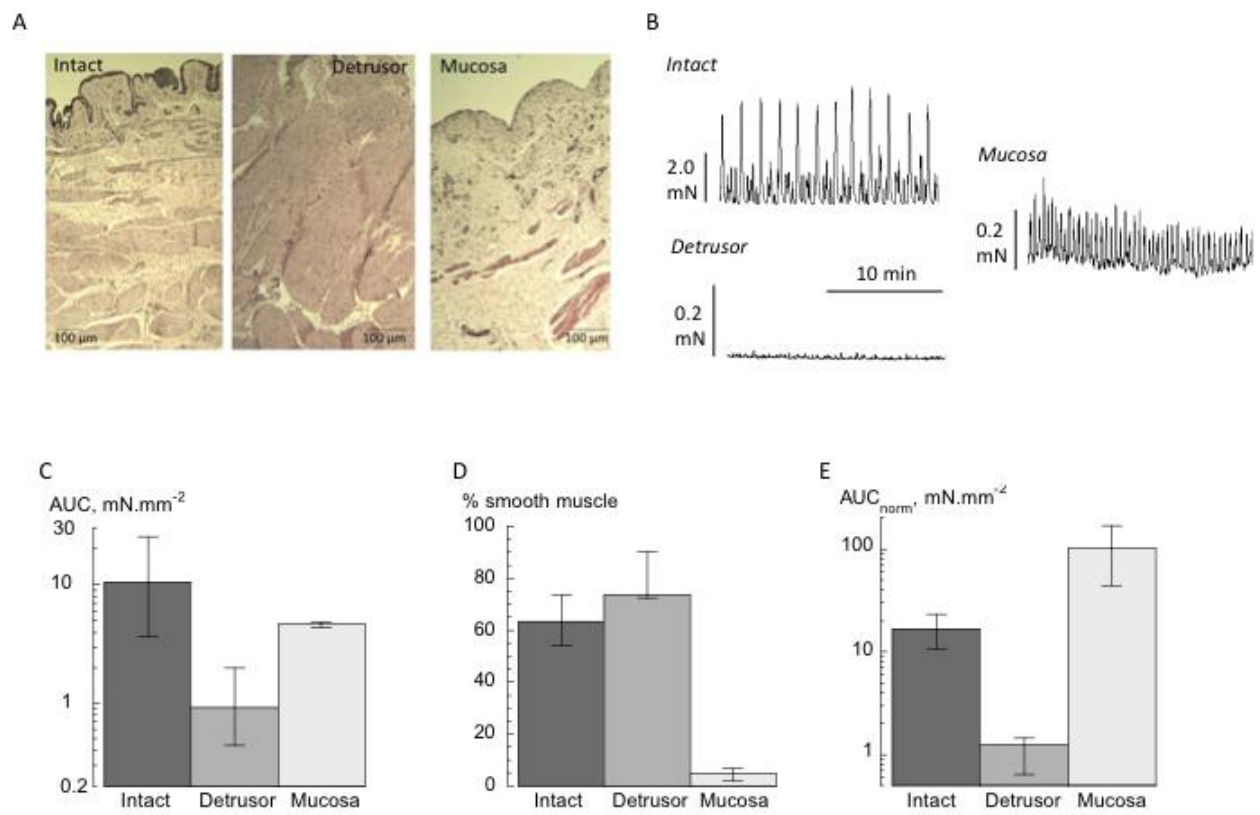


Figure 2

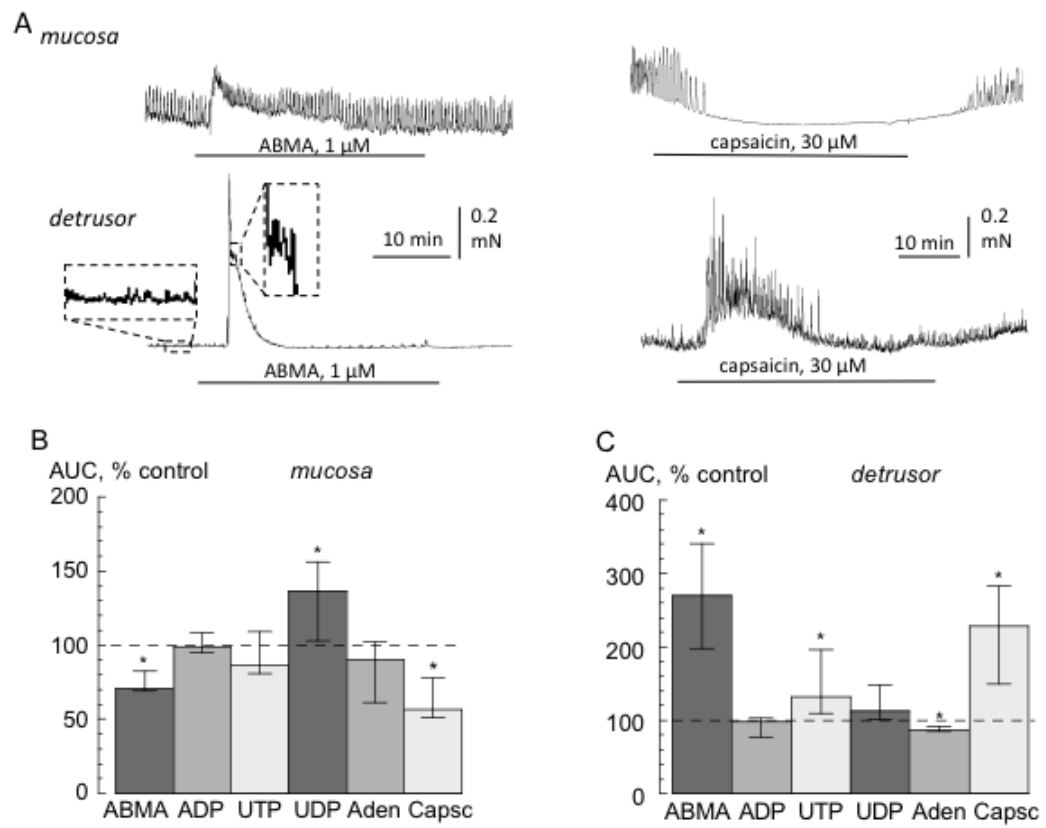


Figure 3

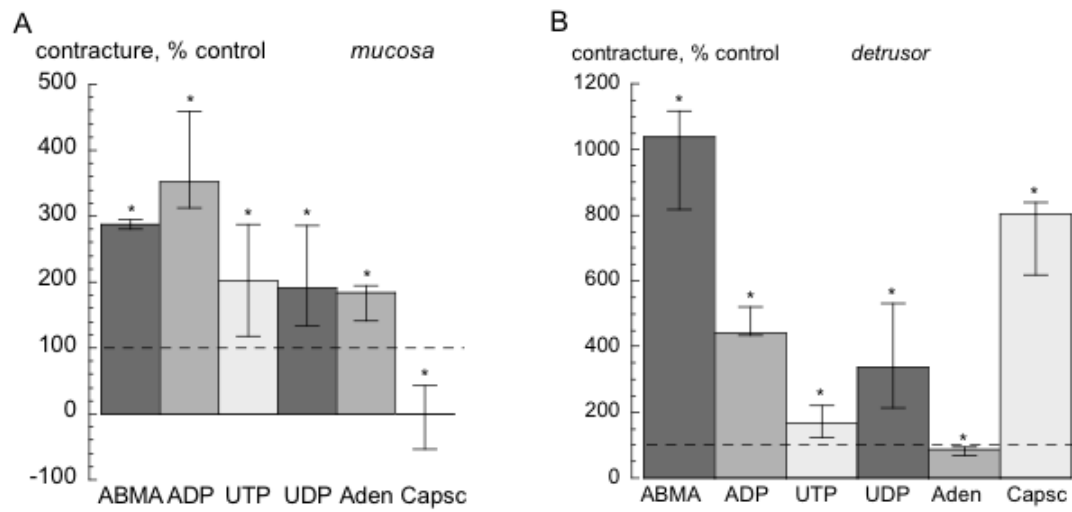


Figure 4

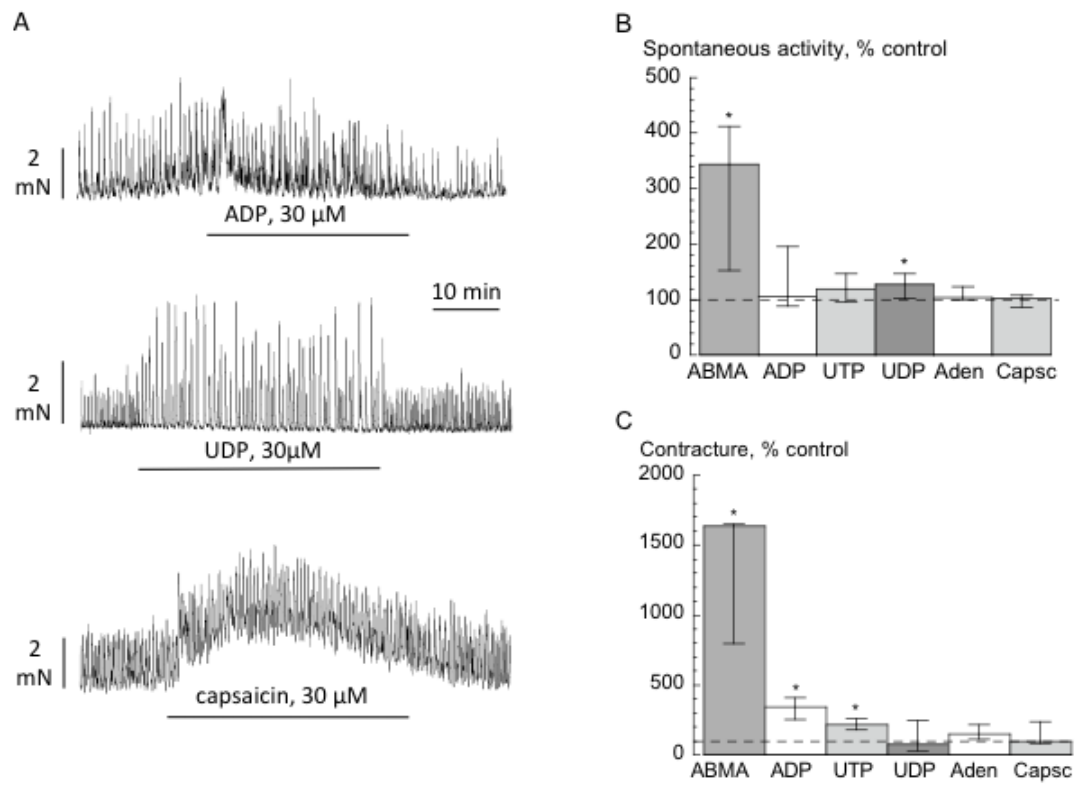


Figure 5

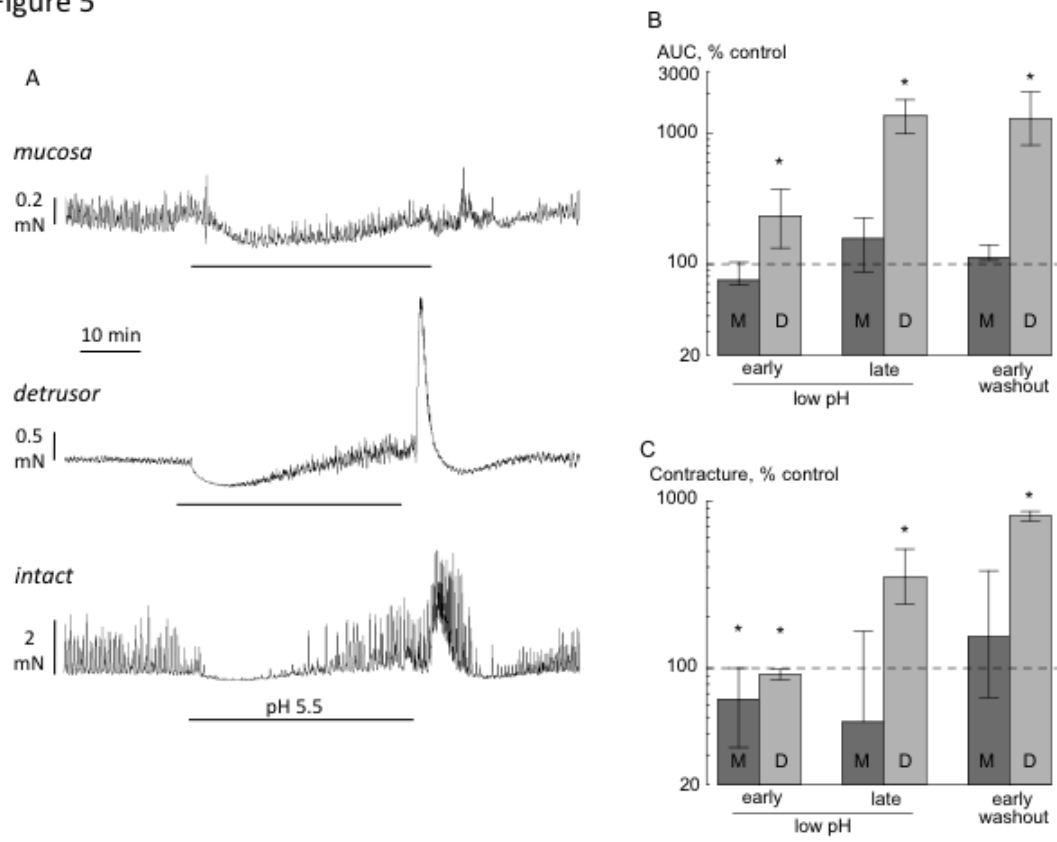
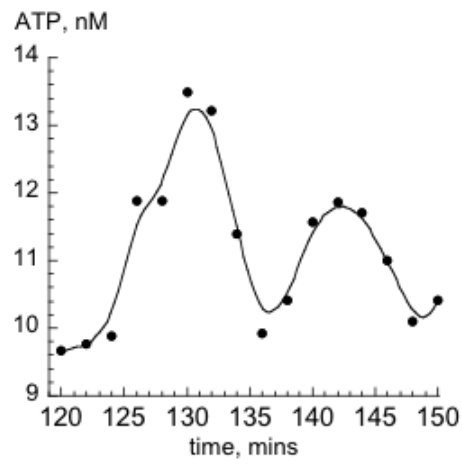


Figure 6 A/B

A



B

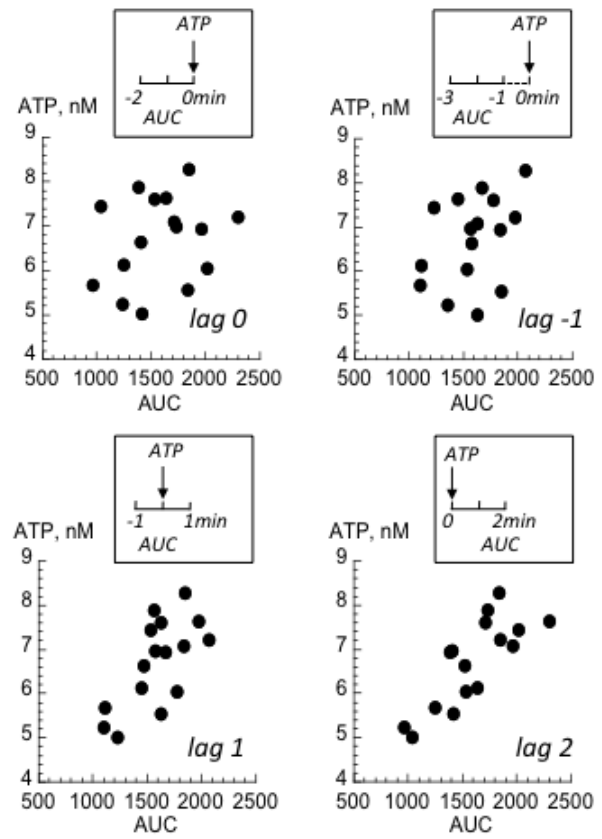
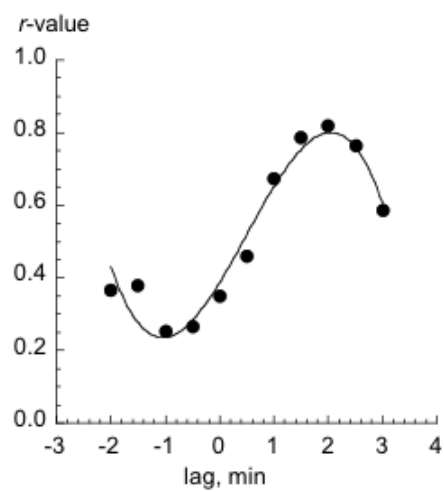


Figure 6 C/D

C



D

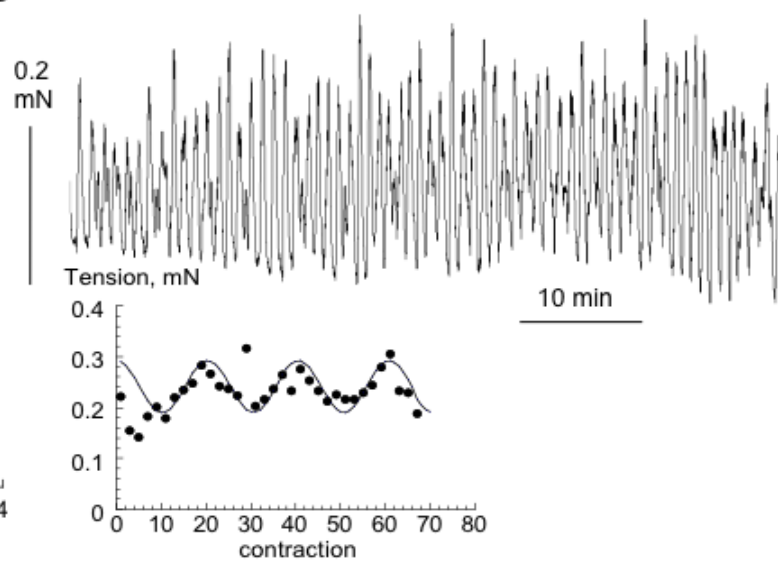


Figure 7

